A comprehensive assessment of the hazards of current use pesticides to native freshwater mussels

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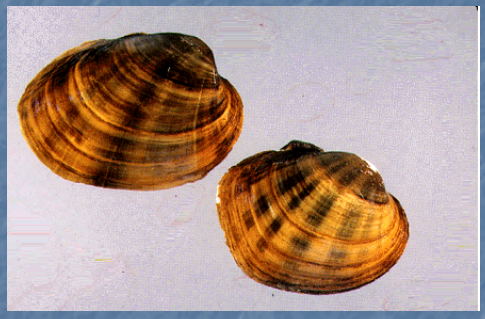
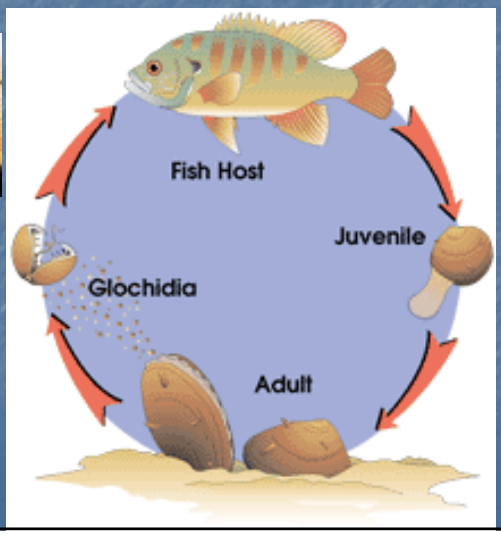
Freshwater mussels
(Family Unionidae)



- Among most imperiled fauna in world
- 70% of 300 species in U.S. are endangered, threatened, of special concern, or extinct
  - Decline due to many environmental stressors
- Long-lived, 40-100 yrs
- Unique life history, obligate parasite on fish

Freshwater mussels: complex life history



Pesticides

- One of many potential factors affecting mussels
- Current-use pesticides not persistent
- But, application often coincides with reproduction
- Early life stages most sensitive to other contaminants
- Paucity of information

Goal

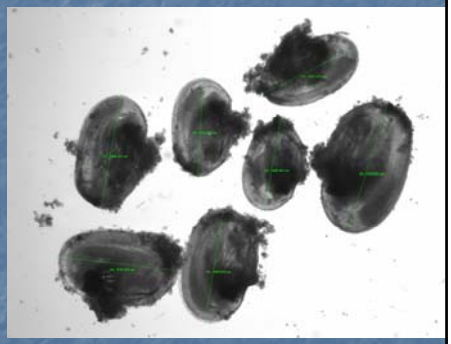
Generate toxicological information on the hazards of a suite of current-use pesticides to early life stages of freshwater mussels

Outline

- Technical grade pesticides
- Glyphosate compounds
- Technical grade vs. formulations
- Summary & conclusions
- Research needs

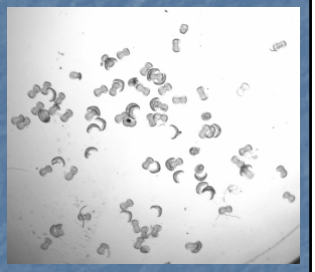
Toxicity test summary

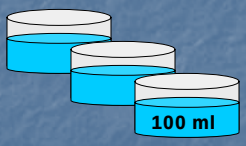
- Total of 86 toxicity tests (2004-2006)
- 73 acute tests
  - 38 glochidia (5 species)
  - 35 juveniles (4 species)
- 13 chronic tests
  - 1 species
- 15 current-use pesticides
  - 5 insecticides
  - 7 herbicides
  - 3 fungicides
  - 2 other (formulation components)



Test methods

- ASTM (2006) Standard guide for toxicity testing with early life stages of freshwater mussels
- 5-6 pesticide concentrations + controls
  - Up to solubility limit in water
  - 3 reps per treatment
- Verify exposure concentration t0
- Acute tests -static
  - Glochidia – 24 or 48 hr (no renewal)
  - Juvenile – 96 hr (renewal @ 48h)
- Chronic tests 21 or 28 d - static
  - Renewal every 48 h
- EC50 – conc. at which viability of organisms was reduced to 50%





Toxicity of technical grade pesticides

Technical grade pesticides

- >98% pure compound; active ingredient
  - EPA pesticide registration
- Atrazine, pendimethalin, fipronil, permethrin, chlorothalonil, propiconazole, pyraclostrobin
- Glochidia
  - *Elliptio complanata, Lampsilis fasciola, L. siliquoidea, Villosa delumbis, V. constricta*
- Juveniles
  - *L. fasciola, L. siliquoidea*
  - Newly released – 2 mo. post transformation
  - Acute tests

Results

- Atrazine, pendimethalin, fipronil, permethrin
- Low or no effect on glochidia & juvenile viability
  - Unable to calculate EC50s

Fungicide toxicity

L. siliquoidea only

	EC50 (mg/L)		LC50 (mg/L)	
	Glochidia 48-hr	Juvenile 96-hr	Fish	Daphnia
Chlorothalonil	0.04	0.28	0.04	0.07
Propiconazole	19.21	10.01	1.5	3.2
Pyraclostrobin	0.08	0.03	0.006	0.016

Toxicity of technical grade pesticides: summary

- Herbicides and insecticides not acutely toxic to mussels
  - Inconsistent with relative sensitivity to some other contaminants
  - Insecticide mode of action- neurotoxicants
- Fungicides highly toxic to mussels
  - Sensitivity similar to other aquatic animals
  - Mode of action is less specific (inhibit ATP production)
- Need to determine chronic toxicity of fungicides for risk assessment

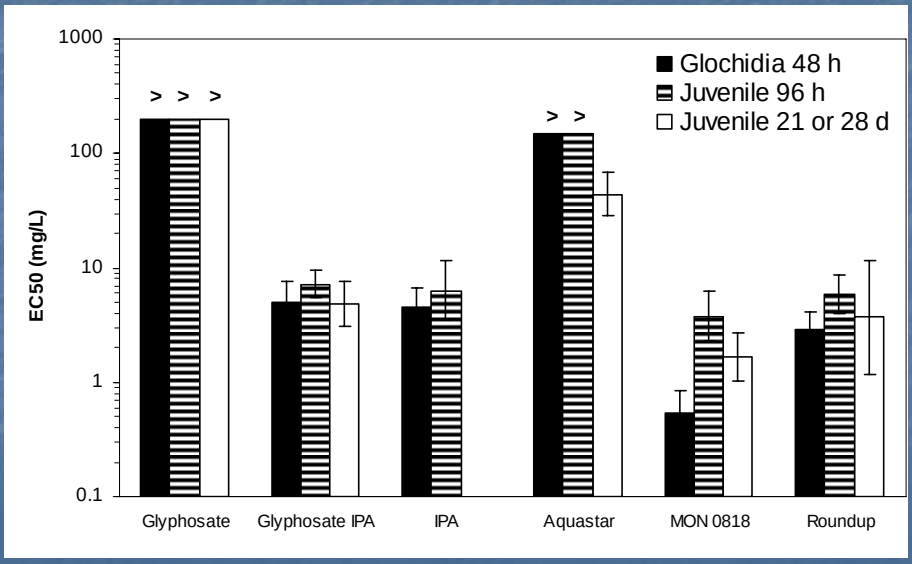
Toxicity of glyphosate

Glyphosate compounds

Chemical	Significance
Technical grade glyphosate	Used for EPA registration
Roundup®	Glyphosate formulation
Aquastar®	Glyphosate formulation (aquatic)
Glyphosate IPA*	A.i. of Roundup® and Aquastar®
MON 0818	Surfactant blend in Roundup®
IPA*	Component of glyphosate IPA

* Isopropylamine salt

Glyphosate toxicity to *L. siliquoidea*



Glyphosate toxicity conclusions

- Technical grade glyphosate not toxic to mussels
- Roundup® is acutely toxic
  - Similar to other aquatic organisms
  - Toxic components
    - Surfactant MON 0818
    - Active ingredient- glyphosate IPA- also toxic
    - IPA toxicity due to ammonia?
- Not all glyphosate IPA compounds are toxic
  - Aquastar®
- Implications for pesticide regulation
  - Register each formulation?

Acute & chronic toxicity of pesticide active ingredients vs. formulations

Technical grade vs. Formulation

Technical grade	Formulation	Class
atrazine	Aatrex 4L®	herbicide
chlorpyrifos	Lorsban®	insecticide
permethrin	Mosquito-B-Gone®	insecticide

Pesticide formulation toxicity

Pesticide	EC50 (mg/L)		
	Glochidia 48 hr	Juvenile 96 hr	Juvenile 21 d
Atrazine	>30	>30	10.1
Aatrex	>30	>30	3.1
Chlorpyrifos	0.43	0.25	0.06
Lorsban	0.60	0.33	0.05
Permethrin	> 0.2	> 0.2	0.03
Mosquito-B-Gone	> 0.2	> 0.2	0.03

Pesticide active ingredient vs. formulations: summary

- Little difference in toxicity of active ingred. & pesticide formulations tested (unlike glyphosate)
- Chlorpyrifos & permethrin caused chronic toxicity at environmentally relevant concentrations
  - *L. siliquoidea* is less sensitive than other aquatic inverts
  - Existing acute water quality criteria may be protective of mussels
- Water only exposures- need to examine other routes of exposure

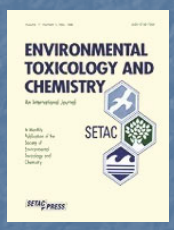
Summary and conclusions

- Early life stages of mussels are not sensitive to many current use pesticides (water-only)
- *L. siliquoidea* highly sensitive to fungicides
- Some pesticide formulations are more toxic to mussels than technical grade; adds to debate about pesticide registration

Research needs

- Fungicide toxicity data for additional species
- Other routes of exposure
  - Role of sediment?
- Effects of pesticides on mussel behavior and physiology

Special Issue of the Journal
Environmental Toxicology & Chemistry
"Ecotoxicology of Unionid Mussels"



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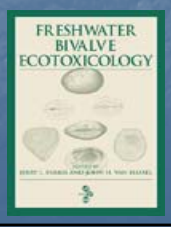

Ten peer-reviewed manuscripts include:

- **Editorial**
- **Intra- and inter-laboratory variation in acute toxicity tests with early life stages**
- **Acute and chronic toxicity of copper, ammonia, and chlorine (water and sediment) to early life stages**
- **Evaluation of derivation of water quality guidance for copper**
- **Ecological risk assessment of copper, ammonia, and chlorine**
- **Acute and chronic toxicity of current use pesticides to early life stages**

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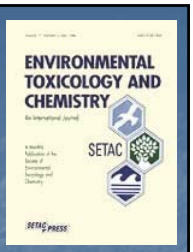


- Bringolf R.B., W.G. Cope, C.B. Eads, P.R. Lazaro, M.C. Barnhart, D. Shea. 2007. Acute and Chronic Toxicity of Technical Grade Pesticides to Glochidia and Juveniles of Freshwater Mussels (Unionidae). Environ. Toxicol. Chem. 26(10):2086-2093.
- Bringolf R.B., W.G. Cope, S. Mosher, M.C. Barnhart, D. Shea. 2007. Acute and Chronic Toxicity of Glyphosate Compounds to Glochidia and Juveniles of *Lampsilis siliquoidea* (Unionidae). Environ. Toxicol. Chem. 26(10):2094-2100.
- Bringolf, R.B., W.G. Cope, M.C. Barnhart, S. Mosher, P.R. Lazaro, D. Shea. 2007. Acute and Chronic Toxicity of Pesticide Formulations (Atrazine, Chlorpyrifos and Permethrin) to Glochidia and Juveniles of *Lampsilis siliquoidea* (Unionidae). Environ. Toxicol. Chem. 26(10):2101-2107.

Funding

Pesticide Environmental Trust Fund

North Carolina Department of Agriculture & Consumer Services

and

U.S. Environmental Protection Agency

Region 5

Family Unionidae
The Freshwater Mussels

- Most imperiled faunal group in world
  - ~300 sp. in N.A.
  - ~67% endangered, threatened, of special concern, or extinct
- Decline since 1800s
  - hastened past 50 yrs
  - numerous factors
  - biological & physiological

Chronic toxicity of glyphosate to juvenile *L. siliquoidea*

Chemical	21 or 28 d EC50 (mg/L)	Growth LOEC* (mg/L)
Glyphosate	> 200	25
Glyphosate IPA	4.8	6.3
Roundup	3.7	5.0
Aquastar	> 200	100
MON 0818	1.7	1.3

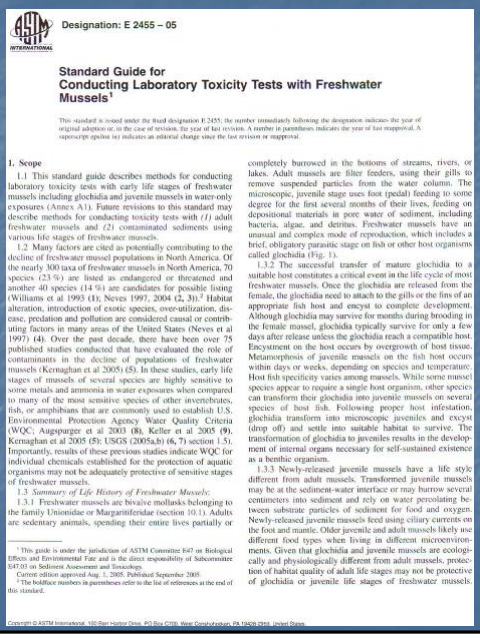
* Lowest Observed Effect Concentration

Chronic toxicity

Pesticide	21-d EC50 (mg/L)	Growth LOEC* (mg/L)
Atrazine	10.1	15
Aatrex	3.1	3.8
Chlorpyrifos	0.06	0.06
Lorsban	0.05	0.05
Permethrin	0.03	0.012
Mosquito-B-Gone	0.03	0.05

* Lowest observed effect concentration

ASTM Guideline



Designation: E 2455 – 05

Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels[1]

This standard is issued under the fixed designation E 2455; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This standard guide describes methods for conducting laboratory toxicity tests with early life stages of freshwater mussels including glochidia and juvenile mussels in water-only exposures (Annex A1). Future revisions to this standard may describe methods for conducting toxicity tests with *(1)* adult freshwater mussels and *(2)* contaminated sediments using various life stages of freshwater mussels.

1.2 Many factors are cited as potentially contributing to the decline of freshwater mussel populations in North America. Of the nearly 300 taxa of freshwater mussels in North America, 70 species (23 %) are listed as endangered or threatened and another 40 species (14 %) are candidates for possible listing (Williams et al 1993 **(1)**; Neves 1997, 2004 **(2, 3)**.[2] Habitat alteration, introduction of exotic species, over-utilization, disease, predation and pollution are considered causal or contributing factors in many areas of the United States (Neves et al 1997) **(4)**. Over the past decade, there have been over 75 published studies conducted that have evaluated the role of contaminants in the decline of populations of freshwater mussels (Kernaghan et al 2005) **(5)**. In these studies, early life stages of mussels of several species are highly sensitive to some metals and ammonia in water exposures when compared to many of the most sensitive species of other invertebrates, fish, or amphibians that are commonly used to establish U.S. Environmental Protection Agency Water Quality Criteria (WQC; Augspurger et al 2003 **(8)**, Keller et al 2005 **(9)**, Kernaghan et al 2005 **(5)**; USGS (2005a,b) **(6, 7)** section 1.5). Importantly, results of these previous studies indicate WQC for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels.

1.3 *Summary of Life History of Freshwater Mussels:*

1.3.1 Freshwater mussels are bivalve mollusks belonging to the family Unionidae or Margaritiferidae (section 10.1). Adults are sedentary animals, spending their entire lives partially or completely burrowed in the bottoms of streams, rivers, or lakes. Adult mussels are filter feeders, using their gills to remove suspended particles from the water column. The microscopic, juvenile stage uses foot (pedal) feeding to some degree for the first several months of their lives, feeding on depositional materials in pore water of sediment, including bacteria, algae, and detritus. Freshwater mussels have an unusual and complex mode of reproduction, which includes a brief, obligatory parasitic stage on fish or other host organisms called glochidia (Fig. 1).

1.3.2 The successful transfer of mature glochidia to a suitable host constitutes a critical event in the life cycle of most freshwater mussels. Once the glochidia are released from the female, the glochidia need to attach to the gills or the fins of an appropriate fish host and encyst to complete development. Although glochidia may survive for months during brooding in the female mussel, glochidia typically survive for only a few days after release unless the glochidia reach a compatible host. Encystment on the host occurs by overgrowth of host tissue. Metamorphosis of juvenile mussels on the fish host occurs within days or weeks, depending on species and temperature. Host fish specificity varies among mussels. While some mussel species appear to require a single host organism, other species can transform their glochidia into juvenile mussels on several species of host fish. Following proper host infestation, glochidia transform into microscopic juveniles and excyst (drop off) and settle into suitable habitat to survive. The transformation of glochidia to juveniles results in the development of internal organs necessary for self-sustained existence as a benthic organism.

1.3.3 Newly-released juvenile mussels have a life style different from adult mussels. Transformed juvenile mussels may be at the sediment-water interface or may burrow several centimeters into sediment and rely on water percolating between substrate particles of sediment for food and oxygen. Newly-released juvenile mussels feed using ciliary currents on the foot and mantle. Older juvenile and adult mussels likely use different food types when living in different microenvironments. Given that glochidia and juvenile mussels are ecologically and physiologically different from adult mussels, protection of habitat quality of adult life stages may not be protective of glochidia or juvenile life stages of freshwater mussels.

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.03 on Sediment Assessment and Toxicology.
Current edition approved Aug. 1, 2005. Published September 2005.
[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.